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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Taka-Aki Sato Examiner: J. E. Goldberg

Serial No.: 09/327,750 Art Unit: 1655

Filed : June 7, 1999

For : GENE ENCODING NADE, $P75^{NTR}$-ASSOCIATED CELL DEATH EXECUTOR AND USES THEREOF

1185 Avenue of the Americas
New York, New York 10036
October 9, 2001

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

**AMENDMENT IN RESPONSE TO SEPTEMBER 7, 2001 COMMUNICATION REGARDING NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES**

This Amendment is submitted in response to the September 7, 2001 Communication Regarding Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures issued by the United States Patent and Trademark Office in connection with the above-identified application. A copy of the Notice to Comply is attached hereto as **Exhibit A.** A response to the September 7, 2001 Notice is due October 7, 2001. However, October 7, 2001 falls on a Sunday and Monday, October 8, 2001 is a federal holiday, i.e. Columbus Day. Therefore, a response filed on the next business day, i.e., Tuesday, October 9, 2001, is considered timely. Accordingly, this Amendment is being timely filed.

Please amend the subject application as follows:

**In the Specification:**

Please insert into the specification the Sequence Listing annexed hereto as Exhibit B.

Please amend the paragraphs of the specification identified below with the amended versions thereof as follows:

The paragraph on page 11, lines 3-10:

**Figure 1A**

Amino acid alignment of mouse (SEQ. ID NO:12) and human NADE (HGR74) (4) proteins (SEQ. ID NO:13). The dotted sequence is asparagine rich stretch. The asterisks indicate the leucine-rich nuclear export signal (NES)(5). The closed triangle indicates cysteine residue essential for dimer formation. The prenylation sequence in C-termini is underlined.

The paragraph on page 11, lines 12-19:

**Figure 1B**

Comparison of leucine-rich nuclear export signal (NES) (5) in various protein. The consensus sequence for NES are shadowed. Genbank accession numbers are: cZyxin, X69190 (SEQ. ID NO:14); MAPKK, D13700 (SEQ. ID NO:15); PKI-a, L02615 (SEQ. ID NO:16); TFIIIA, M85211 (SEQ. ID NO:17); RevHIV-1, AF075719 (SEQ. ID NO:18); RanBP1, L25255 (SEQ. ID NO:19); FMRP, L29074 (SEQ. ID NO:20); Gle1, U68475 (SEQ. ID NO:21); RexHTLV-1 ((SEQ. ID NO:22); Human NADE (SEQ. ID NO:23), submitted; mouse NADE (SEQ. ID NO:24), submitted.

The paragraph on page 11, lines 21-22:

**Figure 1C**

Consensus sequence of ubiquitination signal, Mouse (SEQ. ID NO:25); Human (SEQ. ID NO:26) and Consensus (SEQ. ID NO:27).

The paragraph on page 12, lines 10-13:

**Figure 1G-1 and 1G-2**

Blast Search and comparison of mouse NADE nucleic acid sequence Figure 1G-1 (SEQ. ID NO:28) and human protein HGR74 sequence (SEQ. ID NO:29).

The paragraph on page 12, lines 15-18:

**Figure 1H**

Comparison of mouse NADE, human HGR74 protein and other homologous rat, mouse and human amino acid sequences: musnade3a (SEQ. ID NO:30); hunade3a1 (SEQ. ID NO:31); hunade3a2 (SEQ. ID NO:32); ratnad3a (SEQ. ID NO:33); ratnad3b (SEQ. ID NO:34); musnade3b (SEQ. ID NO:35); humnade1 (SEQ. ID NO:36); ratnade1 (SEQ. ID NO:37); musnade1 (SEQ. ID NO:38); humnade2 (SEQ. ID NO:39).

The paragraph on page 14, lines 31-35:

**Figure 4A**

At residues 88-100, the mouse NADE NES (SEQ. ID NO:40) lies within the C-terminus. A mouse NADE (SEQ. ID NO:41) is aligned with homologous sequences of NADE family members and the NES sequences of HIV Rev (SEQ. ID. NO:42), MAPKK (SEQ. ID NO:43), cZyxin (SEQ. ID NO:44) and PKI-a (SEQ. ID NO:45).

The paragraph on page 16, line 36 through page 17, line 22:

This invention provides an isolated nucleic molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described isolated nucleic molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a DNA molecule. In another embodiment of the above described isolated nucleic acid molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated

nucleic acid is a cDNA molecule. In a further embodiment of the above described isolated DNA molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a RNA molecule. In an embodiment of the above described isolated nucleic acid molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, the isolated nucleic acid is operatively linked to a promoter of RNA transcription. In yet another embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule encodes a neurotrophin associated cell death executor protein. In an embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule comprises a sequence of AATTG TCTAC GCATC CTTAT GGGGG AGCTG TCTAA C (SEQ. ID NO:1).

The paragraph on page 19, line 17 through page 20, line 11:

This invention provides a vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, operatively linked to a promoter of RNA transcription. In an embodiment of the invention, where in the vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, operatively linked to a promoter of RNA transcription is a plasmid. In another embodiment the above described isolated nucleic acid molecule which is a cDNA molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, encodes a human or mouse protein. In yet another embodiment the above described isolated nucleic acid molecule is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor comprising the amino acid sequence set forth in Figure 1A (SEQ. ID NO:13). In a further embodiment the above described isolated nucleic acid molecule is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$

receptor. In an embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor which is a mouse, rat or human protein. In yet another embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule, said isolated nucleic acid molecule comprises the nucleic acid sequence set forth in Figure 1G-1 (SEQ. ID NO:29).

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The paragraph on page 25, lines 5-32:

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This invention provides a purified a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described purified polypeptide capable of binding $p75^{NTR}$ receptor is encoded by the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment the above described polypeptide capable of binding a $p75^{NTR}$ receptor is a fragment of the purified polypeptide capable of binding a $p75^{NTR}$ receptor. In another embodiment the above described purified polypeptide capable of binding a $p75^{NTR}$ receptor has substantially the same amino acid sequence as set forth in Figure 1A (SEQ. ID NO:13). In a further embodiment the above described purified polypeptide capable of binding a $p75^{NTR}$ receptor having an amino acid sequence as set forth in Figure 1A (SEQ. ID NO:13). In yet another embodiment the above described polypeptide capable of binding a $p75^{NTR}$ receptor has an amino acid sequence as set forth in Figure 1A (SEQ. ID NO:13). In a further embodiment, the above described polypeptide capable of binding a $p75^{NTR}$ receptor is a vertebrate polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described polypeptide capable of binding a $p75^{NTR}$ receptor comprises a neurotrophin associated cell death executor protein. In yet another embodiment of the above described polypeptide capable of binding a $p75^{NTR}$ receptor

comprises NCLRILMGELSN (SEQ. ID NO:2).

The paragraph on page 26, line 1-9:

As used herein, a polypeptide capable of binding a $p75^{NTR}$ receptor having "substantially the same" amino acid sequences as set forth in Figure 1A (SEQ. ID NO:13) is encoded by a nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, said nucleic acid having 100% identity in the homeodomain regions, that is those regions coding the protein, and said nucleic acid may vary in the nucleotides in the non-coding regions.

The paragraph on page 26, line 29 through page 20, line 1:

This invention provides a polyclonal antibody directed to an epitope of the purified protein having the amino sequence as set forth in Figure 1A (SEQ. ID NO:13). In a further embodiment the above described monoclonal or polyclonal antibodies are directed to the polypeptide capable of binding a $p75^{NTR}$ receptor, having the amino sequence as set forth in Figure 1A(SEQ. ID NO:13).

The paragraph on page 59, line 35 through page 60, line 34:

**DNA construction.**

A full length mouse NADE cDNA was constructed on pBluescript II vector by the ligation of the partial NADE cDNA (7-524) and 5'-RACE product. PCR cloning techniques were used to replace the stop codon and add the 5' *Xho*I site and 3' *Bam*HI site of a full length NADE cDNA. pcDNA3.1(-)Myc-HisA/NADE was constructed by insertion of a full length NADE cDNA to *Xho*I-*Bam*HI site of pcDNA3.1(-)Myc-HisA (Invitrogen). Human NADE cDNA was amplified using a Jurkat T cell cDNA library and cloned to pcDNA3.1(-)Myc-HisA pcDNA3/rat $p75^{NTR}$ was constructed by insertion of a full

length rat $p75^{NTR}$ cDNA to *Eco*RI site of pcDNA3(Invitrogen). pGEX4T-1/rat $p75^{NTR}$ICD was constructed by insertion of amplified rat $p75^{NTR}$ICD(a. a. 338-396) to pGEX4T-1(Pharmacia). Mutant NADE expression plasmids, pcDNA3.1(-)Myc-HisA/muNADE (Cys102Ser) and pcDNA3.1(-)Myc-HisA/muNADE(Cys121Ser), were constructed by PCR-based site-direct mutagenesis methods (29). pELAM-Lu for luciferase reporter assay was constructed by insertion of NF-κB binding site of E-selectin promoter region (-730 - 52) to pGL3-Basic *Sac*I-*Bgl*II site. Expression plasmids of GFP-fused NADE proteins were made following: The cDNA of GFP was cloned into NheI-XhoI-cut pcDNA3.1-mouse NADE as a PCR product amplified with the primers 5"-CTAGCTAGCATCATGGTGAGCAAGGGCGAG-3" (SEQ. ID NO:3) and 5"-CCGCTCGAGTCTTGTACAGCTCGTCCAT-3" (SEQ. ID NO:4) using pEGFP-N2 (Clontech) as a template. The deletion mutants delta 101-124-GFP and delta 91-124-GFP were constructed by inserting an XhoI-BamHI-cut PCR fragment generated with Expand high fidelity Taq polimerase (Boehringer Mannheim) into XhoI-BamHI-cut pcDNA3.1-GFP using the primers
5"-ATCCTCGAGCGATCATGGCCAATGTCCAC-3" (sense)(SEQ. ID NO:5),
5"-ATCGGATCCTCTCAGCTGTAGCTCCCT-3" (antisense) (SEQ. ID NO:6) and
5"-ATCGGATCCGATCTCTCTCATCTCCTC-3" (antisense)(SEQ. ID NO:7).

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The paragraph on page 60, line 36 through page 61, line 6:

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The mutagenic primers
(5'-AAAGCTTAGGGAGGCACAGCTGAGAAA-3" (SEQ. ID NO:8),
5"-TTTCTCAGCTGTGCCTCCCTAAGCTTT-3" (SEQ. ID NO:9),
5"-ATCCGGAGAAAGGCTAGGGAGGCACA-3" (SEQ. ID NO:10),
and 5"-TGTGCCTCCCTAGCCTTTCTCCGGAT-3")(SEQ. ID NO:11)
were used to obtain L97A-GFP and L94, 97A-GFP in which Leu94 and Leu97 are replaced with Ala. In all constructs, mutations were verified by sequencing.

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**REMARKS**

In the September 7, 2001 Notice, the Examiner stated that the subject application does not comply with the requirements set forth in 37 C.F.R. §§ 1.821 - 1.825 because the previously submitted copy of the "Sequence Listing" in computer readable form does not comply with the requirements of §1.822 and/or §1.823.

Specifically, the Examiner required applicant to provide 1) a substitute computer readable form (CRF) copy of the "Sequence Listing"; 2) a substitute paper copy of the "Sequence Listing", and 3) a statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. §1.821(e), §1.821(f), §1.821(g), §1.825(b) or §1.825(d).

In response, applicant submits herewith a substitute computer readable form (CRF) of the "Sequence listing" in ASCII (DOS) format on the enclosed computer diskette.

Applicant further submits a paper copy of the Sequence Listing, attached hereto as **Exhibit B**, and a Statement of Compliance Under 37 C.F.R. §1.821(f) attached hereto as **Exhibit C**, certifying that the computer readable form as required by 37 C.F.R. §1.821(e) is identical to the paper copy of the Sequence Listing attached as **Exhibit B**. Applicant believes that the enclosed substitute C.R.F., the paper copy of the Sequence Listing (**Exhibit B**) and Statement of Compliance Under 37 C.F.R. §1.821(f)) (**Exhibit C**) now fully comply with the requirements of §1.821 through 1.825.

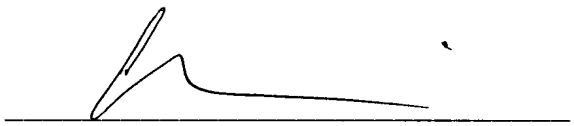
Pursuant to the requirements of 37 C.F.R. §1.121, applicant

annexes hereto as Exhibit D those paragraphs amended herein marked up to show the changes made herein relative to the previous versions thereof.

If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicant's undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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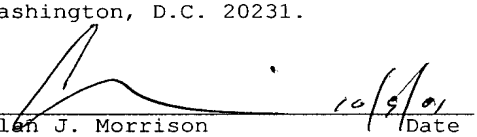

10/9/01
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